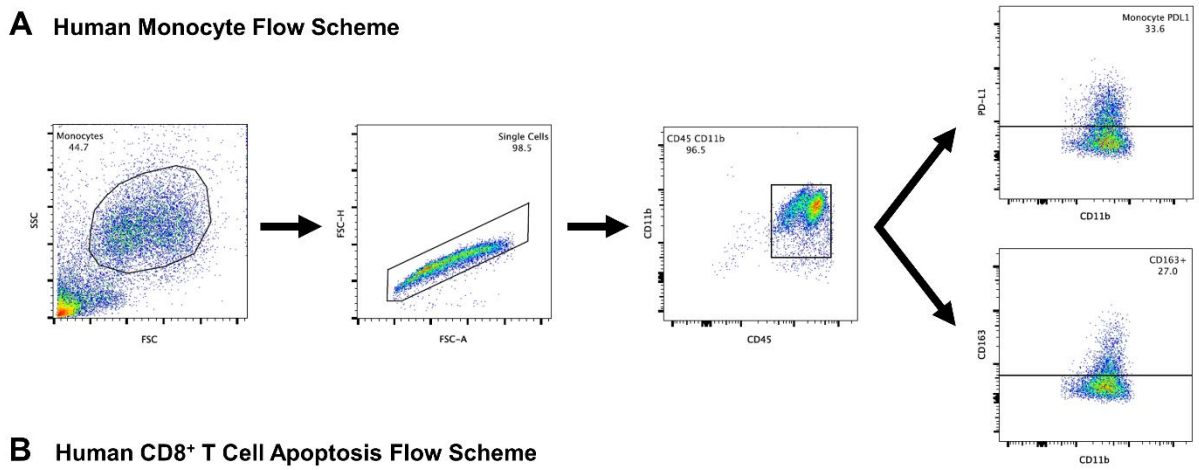
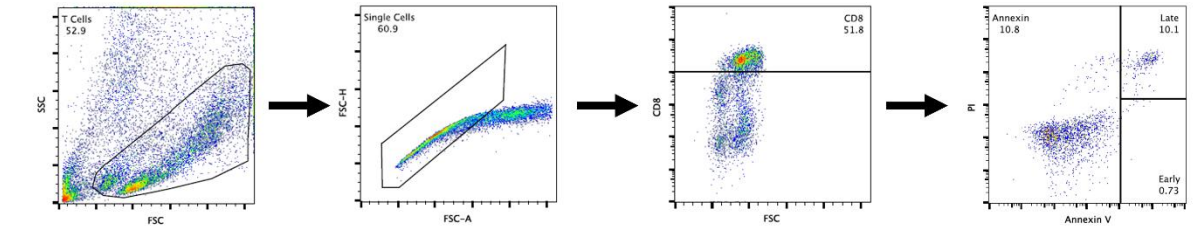
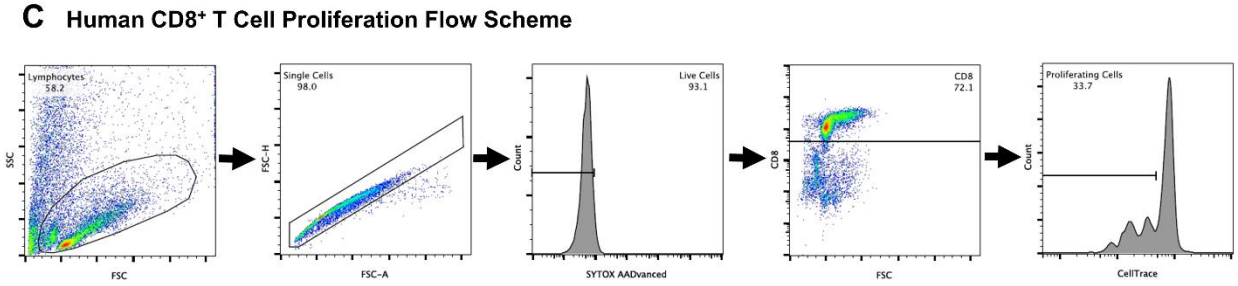
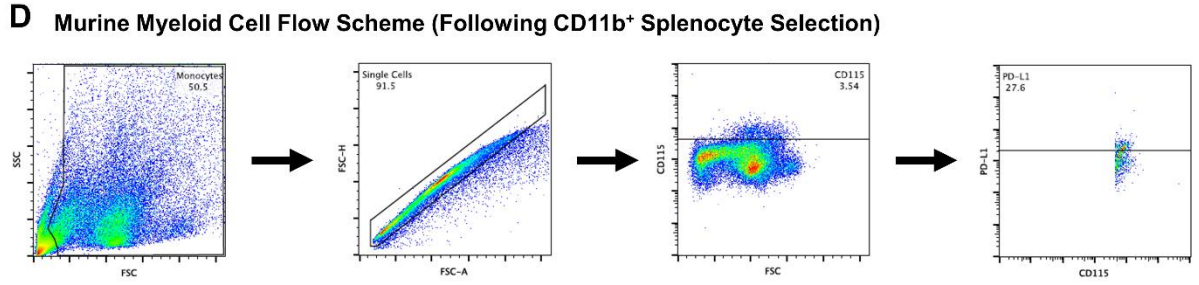
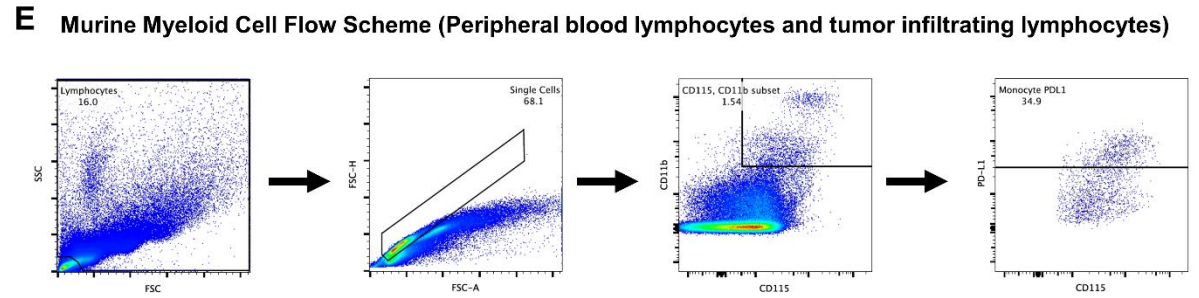
Supplementary Figures



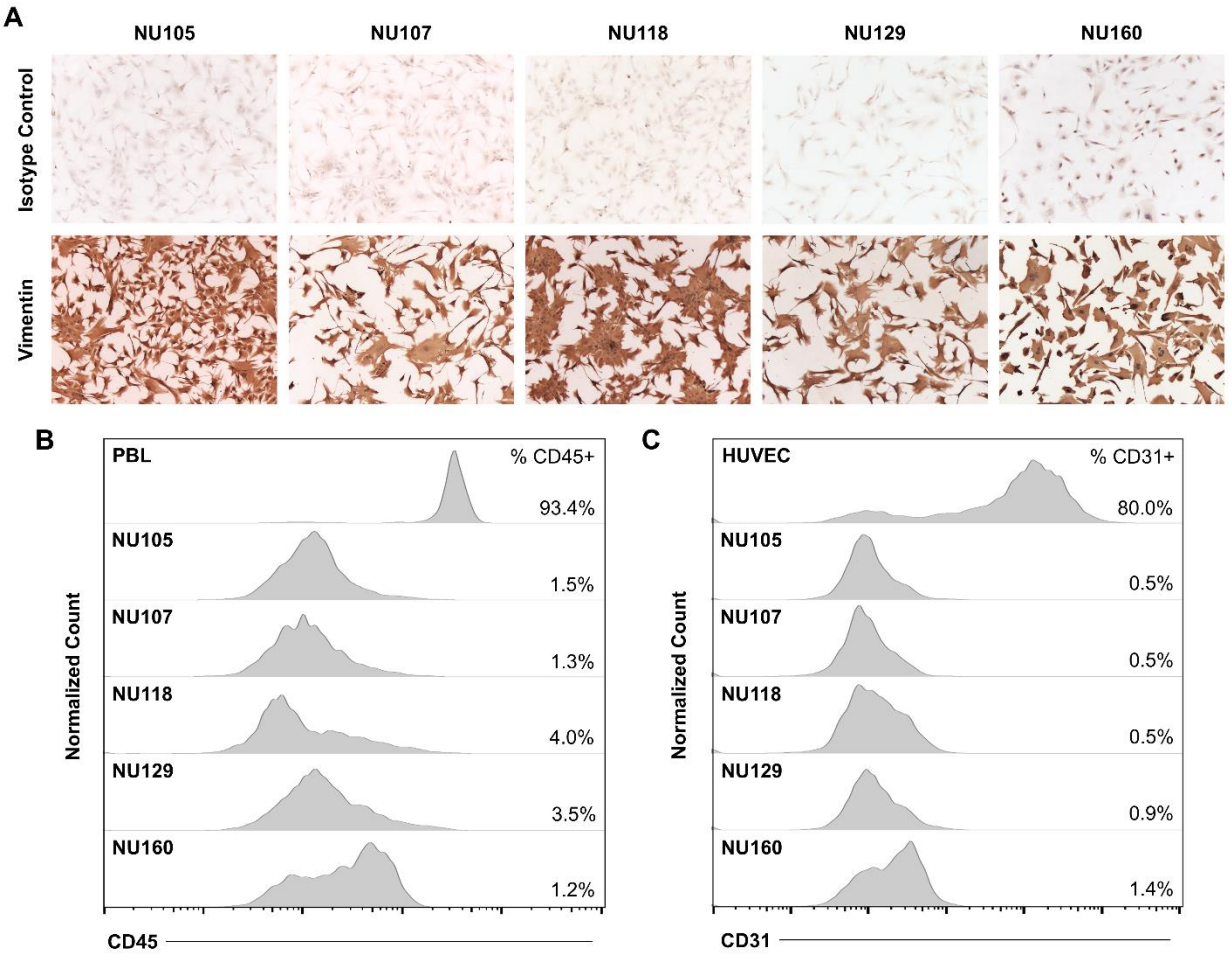




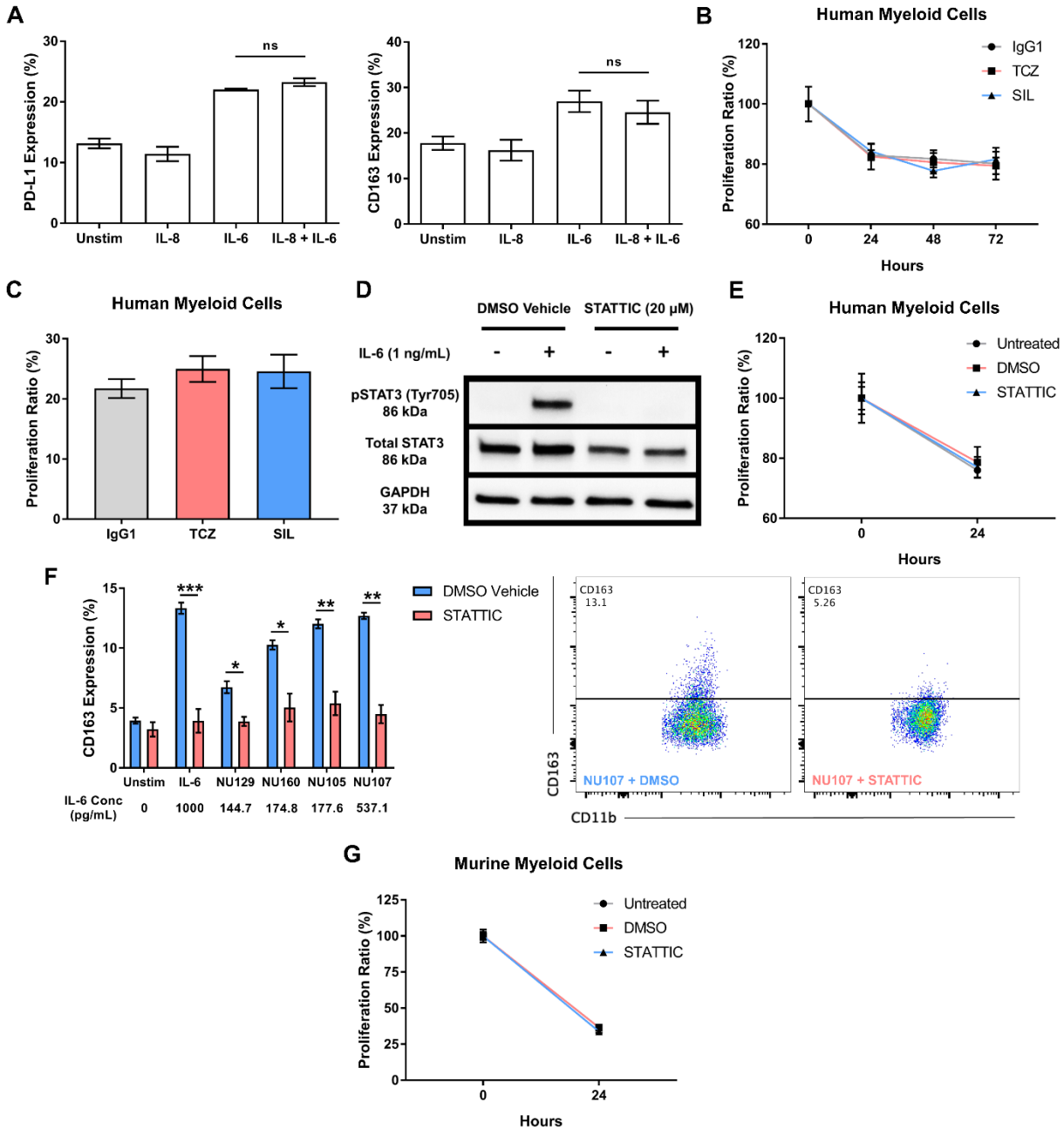




Supplementary Figure S1. Representative flow cytometry gating schemes. Representative flow cytometry gating schemes are presented for the following experiments: (**A**) human $CD45^+$ $CD11b^+$ monocyte PD-L1 and CD163 expression, (**B**) human $CD8^+$ T cell apoptosis, (**C**) human $CD8^+$ T cell proliferation, (**D**) murine $CD115^+$ PD-L1 expression following $CD11b^+$ splenocyte selection, and (**E**) murine $CD11b^+$ $CD115^+$ PD-L1 expression from peripheral blood and tumor infiltrating lymphocytes.

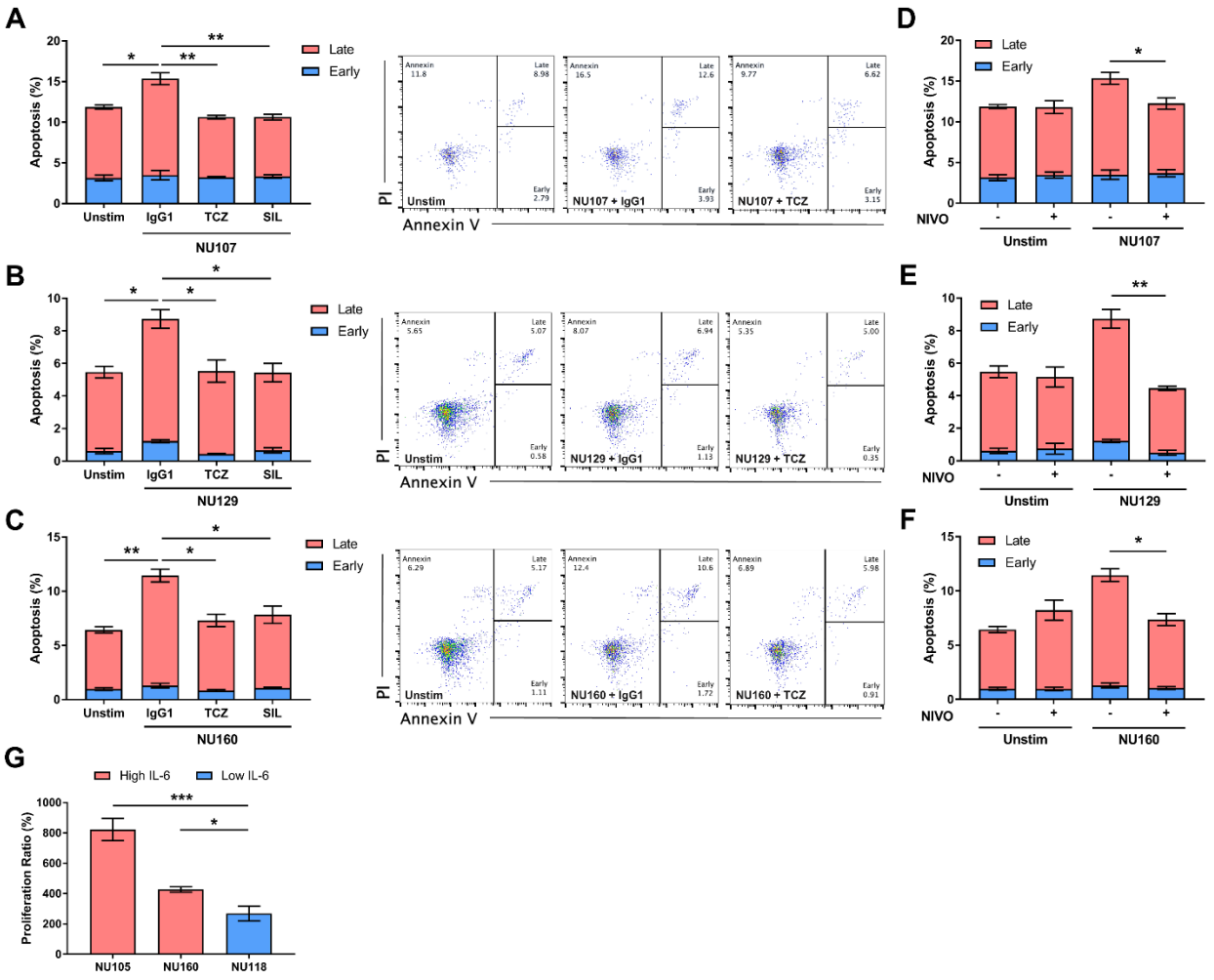


Supplementary Figure S2. GBM explant cell culture characterization. A, GBM explant cell cultures stained positive for the tumor marker vimentin and demonstrated astrocytic morphology. **B** and **C,** GBM explant cell cultures stained negative for CD45 and CD31, indicating no contamination by lymphocytes or endothelial cells.



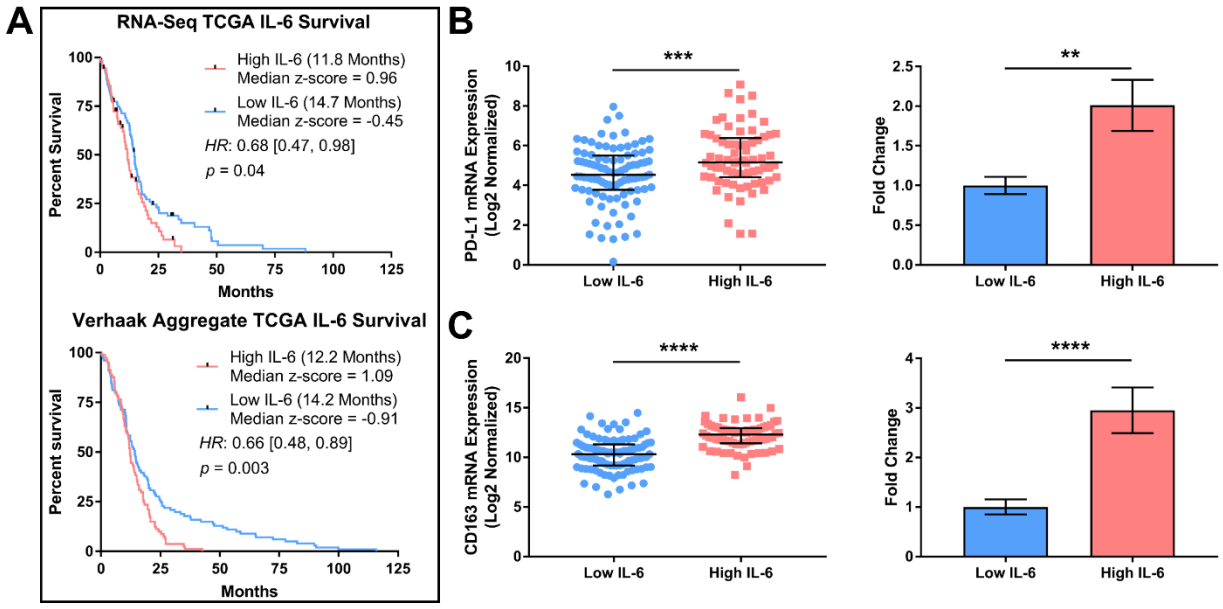
Supplementary Figure S3. Human and murine myeloid cell characterization and viability. A, Combinatorial IL-6 (1 ng/mL) and IL-8 (1 ng/mL) stimulation did not result in an additive increase in PD-L1 or CD163 compared to IL-6 stimulation alone (N=3 replicates per condition). Treatment of human myeloid cells with tocilizumab (TCZ) or siltuximab (SIL) did not negatively affect (**B**) viability (MTT assay) or (**C**) proliferation (BrdU assay) compared to myeloid cells treated with IgG1 isotype control (N=3

replicates per condition). **D,** Treatment of human myeloid cells with STATTIC, an irreversible STAT3 inhibitor was sufficient to inhibit STAT3 phosphorylation induced by IL-6 stimulation. **E,** Moreover, exposure of human myeloid cells to STATTIC or DMSO vehicle control did not negatively affect myeloid cell viability (MTT assay) compared to untreated cells (N=3 replicates per condition). **F,** Inhibition of STAT3 with STATTIC prevented myeloid CD163 induction caused by exposure to IL-6 (P=0.001) and GBM conditioned media (P<0.05; N=3 replicates per condition). **G,** Murine myeloid cells treated with STATTIC or DMSO vehicle control do not exhibit reduced viability compared to untreated cells (N=3 replicates per condition). One-way ANOVA with post-hoc multiple comparisons test was performed for comparisons across experiments with ≥ 3 conditions. Unpaired t-tests were performed for comparisons across 2 conditions. Bars represent the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, ns=non-significant.

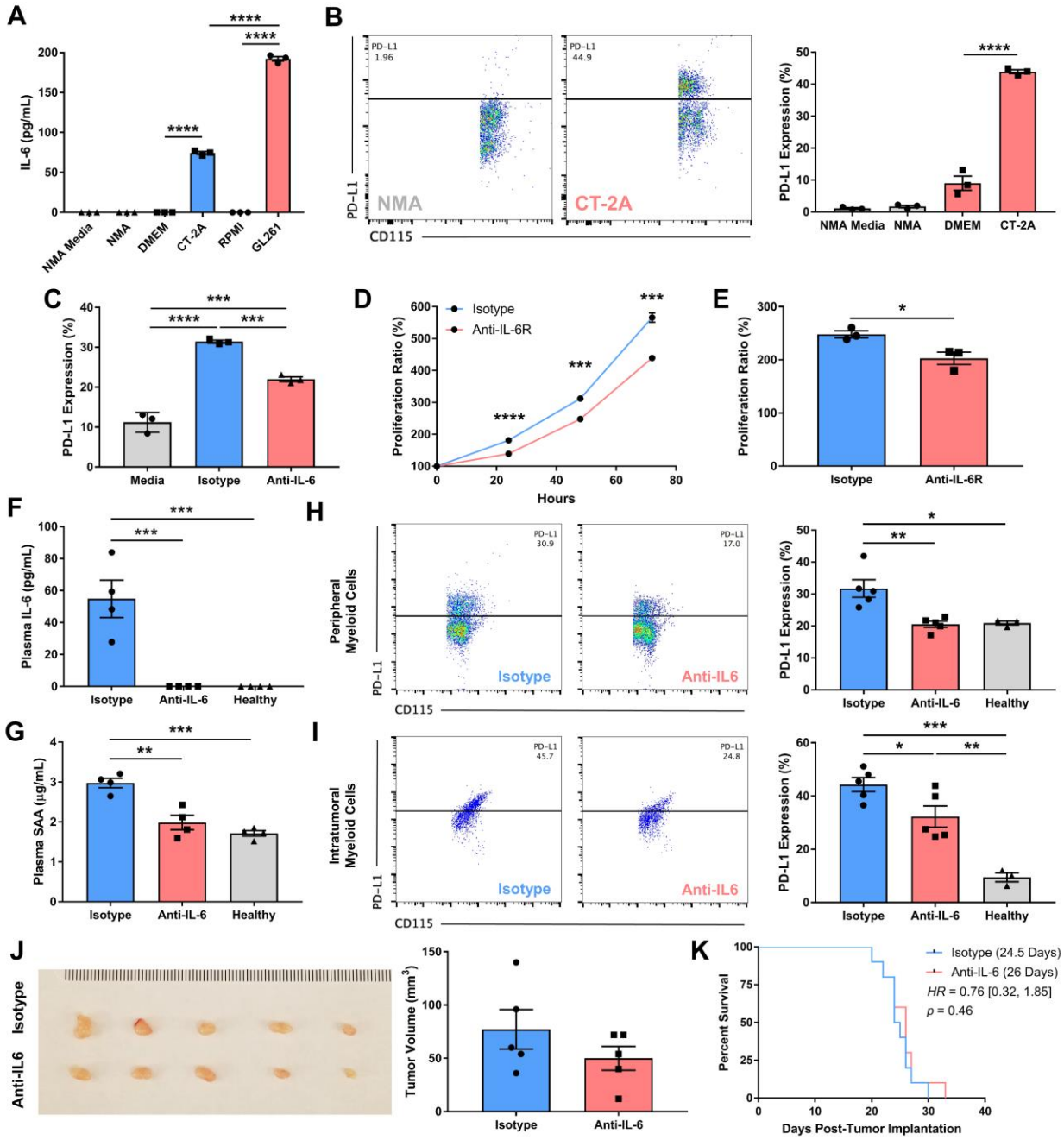


Supplementary Figure S4. GBM-derived IL-6 promotes CD8$^+$ T cell apoptosis through induction of myeloid PD-L1. A through **C,** Myeloid cells stimulated with GBM conditioned media (NU107, NU129, NU160) induce greater CD8$^+$ T cell apoptosis compared to unstimulated myeloid cells (P<0.05; N=3 replicates per condition). The increase in apoptosis could be prevented by treatment of myeloid cells with tocilizumab (TCZ) or siltuximab (SIL) during stimulation with GBM conditioned media (P<0.05; N=3 replicates per condition). **D** through **F,** Treatment of myeloid cell-CD8$^+$ T cell co-cultures with nivolumab (anti-PD-1; NIVO) was also sufficient to reduce CD8$^+$ T cell apoptosis compared to untreated co-cultures (P<0.05; N=3 replicates per condition). **G,** GBM explant cell cultures exhibiting elevated IL-6 expression (NU105 = 177.6 pg/mL; NU160 = 174.8 pg/mL) demonstrated increased proliferation ratios compared to GBM explant cell cultures with lower IL-6 expression (NU118 = 34.5 pg/mL) as measured via BrdU

proliferation assay ($P<0.05$; N=3 replicates per condition). One-way ANOVA with post-hoc multiple comparisons test was performed for comparisons across experiments with ≥ 3 conditions. Bars represent the mean ± SEM. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

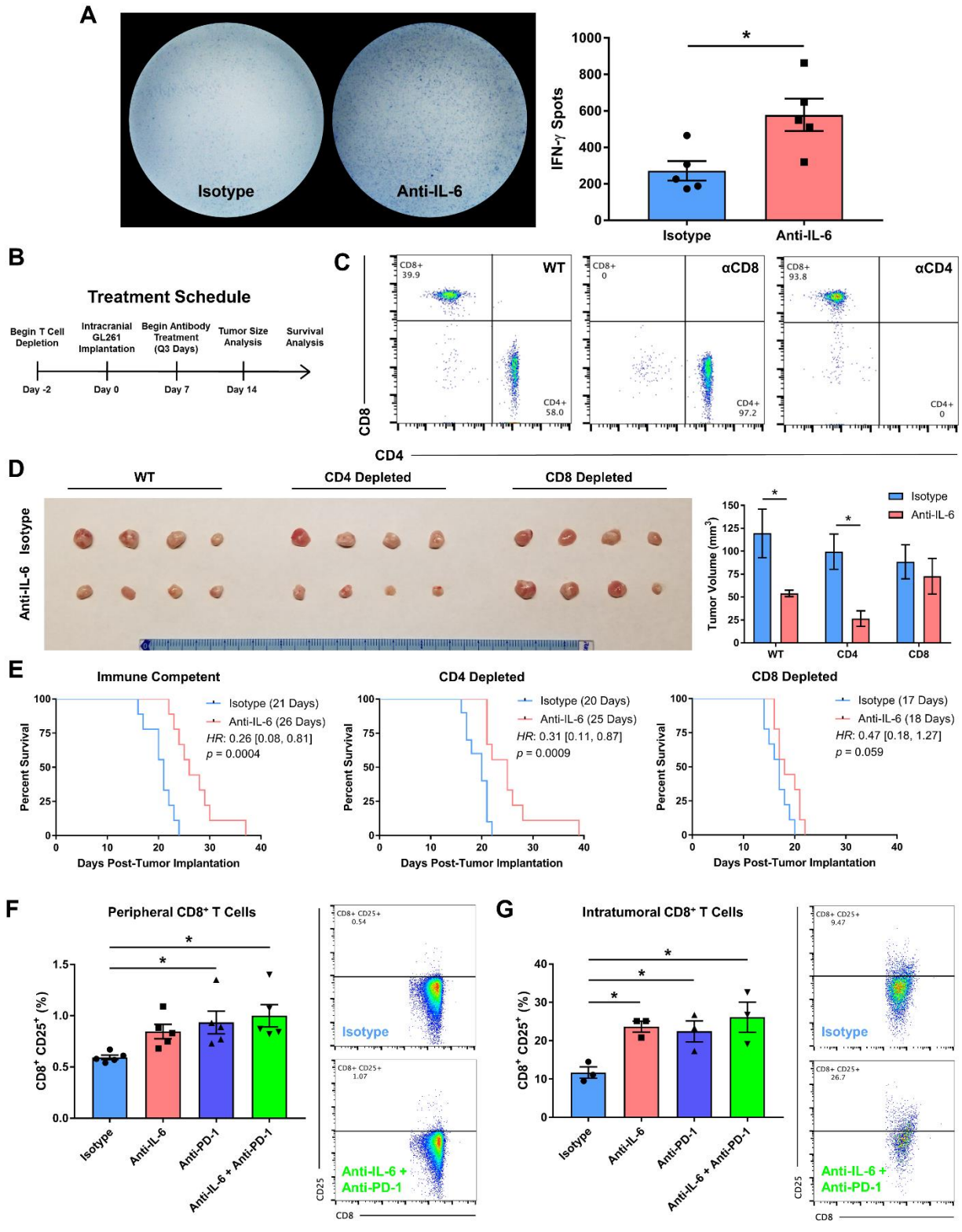


Supplementary Figure S5. *IL6* expression correlates with survival and immunosuppressive markers in GBM. A, TCGA RNA-Seq and microarray data demonstrated that patients with high *IL6* expression experience worse overall survival compared to patients with low *IL6* expression (RNA-Seq *P*=0.04, High *IL6* N=70, Low *IL6* N=95; microarray *P*=0.003, High *IL6* N=81, Low *IL6* N=101). High and low *IL6* expression was determined by positive and negative z-scores, respectively. Patients with elevated *IL6* expression also demonstrated increased expression of immunosuppressive markers (**B**) *PDL1* (*CD274*, *P*=0.0005) and (**C**) *CD163* (*P*<0.0001) within the tumor microenvironment (log2 normalized bars represent the median and interquartile range; fold change bars represent mean ± SEM). Unpaired t-tests were performed to identify differences across groups. Log-rank test was performed to determine survival differences. Hazard radio (HR) reported with 95% confidence interval. ***P*<0.01, ****P*<0.001, *****P*<0.0001.



Supplementary Figure S6. IL-6 is elevated and induces myeloid PD-L1 in the murine CT-2A glioma model. A and **B,** Compared to normal mouse astrocytes (NMA) and media controls, CT-2A cells demonstrated increased IL-6 expression (P<0.0001; N=3 replicates per sample). However, the level of IL-6 was less than GL261 cells (P<0.0001; N=3 replicates per sample). **B,** Myeloid cells exposed to CT-2A CM expressed elevated PD-L1 compared to myeloid cells stimulated with NMA CM or media alone

(*P*<0.0001; N=3 replicates per sample). **C,** Myeloid cell treatment with an IL-6 neutralizing antibody during CT-2A CM stimulation reduced myeloid PD-L1 induction (*P*=0.0009; N=3 replicates per treatment group). CT-2A proliferative capacity was reduced by IL-6R blockade as determined by (**D**) MTT (*P*<0.001) and (**E**) BrdU (*P*=0.03; N=3 replicates per time point) assays. Mice bearing intracranial CT-2A tumors demonstrated increased plasma (**F**) IL-6 and (**G**) SAA that was reduced by treatment with anti-IL-6 (*P*<0.01, N=4 replicates per time point). **H** and **I,** IL-6 inhibition was associated with decreased peripheral (*P*=0.004, N≥3 replicates per conditions) and intratumoral (*P*=0.049; N≥3 replicates per conditions) myeloid cell PD-L1 expression. **J,** Tumors from mice receiving anti-IL-6 treatment demonstrated a non-significantly reduced volume compared to isotype control treated mice (*P*=0.24; N=5 replicates per condition). **K,** No significant difference in survival was observed in CT-2A tumor-bearing mice treated with anti-IL-6 compared to isotype control (*P*=0.46; N=10 isotype control mice, 10 anti-IL-6 mice). One-way ANOVA with post-hoc multiple comparisons test was performed for comparisons across experiments with ≥ 3 conditions. Unpaired t-tests were performed for comparisons across 2 conditions. Log-rank test was performed to determine survival differences. Hazard ratio (HR) reported with 95% confidence interval. Bars represent the mean ± SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.



Supplementary Figure S7. IL-6 inhibition improves T cell function and improves survival in CD8⁺ T cell dependent manner. A, Peripheral T cells from GL261 tumor-bearing mice treated with anti-IL-6

therapy demonstrated greater IFN-γ expression than T cells from mice treated with isotype control (P=0.019, N=5 mice per group, 2 replicates per mouse) as measured by ELISPOT assay. **B** and **C,** To determine the role of $CD4^+$ and $CD8^+$ T cells in the therapeutic benefit of anti-IL-6 therapy, CD4 and CD8 specific T cell depletion was performed prior to GL261 tumor implantation and treatment with anti-IL-6 or isotype control antibodies. **D,** While suppressed tumor growth was observed in immune competent (P=0.049) and CD4 depleted (P=0.01) mice receiving anti-IL-6 treatment, no difference in tumor size was observed in CD8 depleted mice (N=4 replicates per condition). **E,** Similarly, survival benefits were observed in immune competent (P=0.0004) and CD4 depleted (P=0.0009) mice, but not CD8 depleted mice. Compared to GL261 tumor-bearing mice treated with isotype control antibodies, mice treated with anti-IL-6, anti-PD-1, or anti-IL-6 + anti-PD-1 antibodies demonstrated increased $CD8^+$ T cell activation both in the (**F**) peripheral circulation (P<0.05, N=5 replicates per treatment group) and (**G**) within the tumor microenvironment (P<0.05, N=3 replicates per treatment group). One-way ANOVA with post-hoc multiple comparisons test was performed for comparisons across experiments with ≥ 3 conditions. Unpaired t-tests were performed for comparisons across conditions. Log-rank test was performed to determine survival differences. Hazard ratio (HR) reported with 95% confidence interval. Bars represent the mean ± SEM. *P<0.05.